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GREEN SYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTIBACTERIAL ACTIVITY FROM THE LEAVES OF *PUTRANJIVA ROXBURGHII* WALL. (PUTRANJIVAECEAE).

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ABSTRACT
The bio-molecules of various plant components are potentially capable of reducing nanoparticles in their ionic forms. Green synthesis of nanoparticle is the development of simple, inexpensive and, eco-friendly process. This paper presents the green synthesis or bio-synthesis of silver nanoparticles using *Putranjiva roxburghii* Wall., plant leaf extract as the reducing agent. The study also has confirmed the synthesis of nanoparticles are by color change and it was characterisation by UV-visible spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD). Antibacterial activity of biologically synthesized nanopartilcles against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella paratyphi* and *Serretia marcescens* bacteria was proved. This study, therefore has established showed that the *P. roxburghii* leaf extract could as a source for the green synthesis of silver nanoparticles with the appropriate antibacterial activity.

KEYWORDS: *Putranjiva roxburghii* Wall, Silver Nanoparticles, Chhattisgarh, Antibacterial assay.

INTRODUCTION
Green synthesis of nanoparticles is very valuable in the use of nanoparticle as it plays a crucial role in the field of medical science.[1] Research and characterisation has made rapid strides in recent years due to its vast range of application in biology, physics, chemistry and medicine fields.[2] Many techniques are now available as for example chemical reduction of silver ions in aqueous solutions with or without agents.[3] Some of the methods are extremely costly and toxic which may causes potential risks to environment.[4] Plants as well as microbes are now in use for synthesis of nanoparticle. Green synthesis is rapid, cost affordable, eco-friendly and environmentally acceptable.[5] Synthesis by plant-mediated method is much favoured as it is cost-effective, environmentally friendly, and safe for human therapeutic use.[6]

Silver has been recognized for its effective property against microbes in medical and industrial process.[7]; [8] The unique role of silver and silver nanoparticles is in medical industry as ointments to prevent infection against burns and open wounds.[9] It draws attention due to its diverse properties like catalysis, magnetic and optical polarizability.[10] By adoption of various methods there is now the availability broad spectrum of silver nanoparticles. However there is a need to develop eco-friendly procedures to prevent the harmful and toxic chemical synthesis protocol and also to avoid the adverse effects in medical application.[11] Presently there has emerged the application of nanoparticle synthesis in biotechnology with the thrust towards antibacterial and antifungal activities.[12]

Putranjiva roxburghii Wall. is an evergreen tree of 12 m tall, with leaves being simple, alternate, dark green, shiny, elliptic-oblong. They occur in the wild and grow as cultivated trees in almost all parts of India. Traditional use of leaves of *P. roxburghii* Wall. has been in the treatment of skin disease, fever, sterility and also used in cold, fever, and rheumatism.[13] It possess high analgesic, antipyretic, and anti-inflammatory activity.[14]

MATERIALS AND METHODS
Collection and Authentication
The plant was collected from Kunkuri in Jashpur district of Chhattisgarh, India and identified by Dr. S.John Britto, Director and Head, The Rapinat Herbarium and Center for Molecular Systematics St. Joseph's College (Autonomous) Tiruchirappalli, India. The voucher specimen was deposited at the centre with accession number RHT67530.

Preparation of Plant Extract
10 g of dried leaf powder was mixed with 100 ml of deionized water and boiled for 10 minutes. The aqueous extract was then separated by filtration with Whatman No. 1 filter paper (Maidstone, UK) and then centrifuged at 1200 rpm for 5 minutes to remove heavy biomaterials, the extract was then stored at 4°C for further experiments.

Synthesis of AgNPs
A volume of 10 ml of leaf extract was mixed with 90 ml of 1 mM AgNO3 solution. The color change of the solution from yellow to dark green was indicative of the reduction process Ag+ to Ag^0 nanoparticles. As the concentration of AgNO3, increases in the solution the size of the particle also increases.[15]

UV analysis
Synthesized AgNPs were scanned by UV-Vis spectrophotometer at the wavelength of 300-800 nm on Perkin-Elmer Lambda 25 spectrophotometer. It is basically done for monitoring the AgNPs as UV-Vis spectroscopy is used for the characterization of colloidal particles. Noble metal particles possess strong Surface Plasmon Resonance (SPR) absorption in the visible region and are highly sensitive to the surface modification.

X-ray diffraction (XRD) analysis
The particle size and nature of the silver nanoparticle were determined using XRD. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation.
$D = 0.94 \lambda / B \cos \Theta$

Antibacterial assay of AgNPs
The synthesized AgNPs were tested for their antibacterial activity against various microorganisms, *Escherichia coli* (MTCC 40), *Klebsiella pneumonia* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424), *Salmonella paratyphi* (MTCC 3220) and *Serratia marcescens* (MTCC 86) by the disc diffusion method. The pure cultures of the organism were subcultured on nutrients broth. Each strain was swabbed uniformly on the individual plates of nutrient agar using sterile cotton swabs. Size of sterile disc 6 mm diameter were loaded with synthesized AgNPs into all plates and left for incubation at 37ºC for 24 hrs.

RESULTS AND DISCUSSION

UV analysis
The color change was observed from pale yellow to dark green (Fig. 2). The color change is propotional to the extract concentration which indicates the formation of AgNPs, corresponds to the excitation of surface plasmon vibration in the AgNPs after 24 hrs. SPR of AgNPs can be observed is between 220 and 280 nm (Fig. 1).

SEM analysis
SEM (Fig. 4) shows the distribution of AgNPs with *Putranjiva roxburghii* Wall. leaf extract. It showed the uniform shape of nanoparticle formation with diameter range 34-63 nm.

X-ray diffraction (XRD) analysis
The size and structure of the green synthesized nanoparticles were confirmed by the typical XRD patterns and shown in Fig. 3. The XRD pattern indicates the presence of four diffraction peaks are (111), (200), (220) and (311) diffractions.

Antimicrobial properties
There are earlier reports on *P. roxburghii* extracts for its antioxidant[16] and anti-diabetic activities. Synthesized AgNPs was tested for five bacterial strains. Among them highest activity showed against *E.coli* followed by *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Salmonella paratyphi.* (Fig. 5).

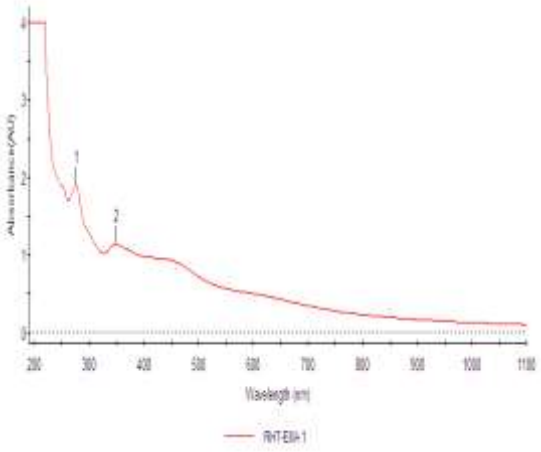

Fig 1: UV spectrum of synthesized AgNPs.

Fig 2: Synthesis of silver nanoparticles.

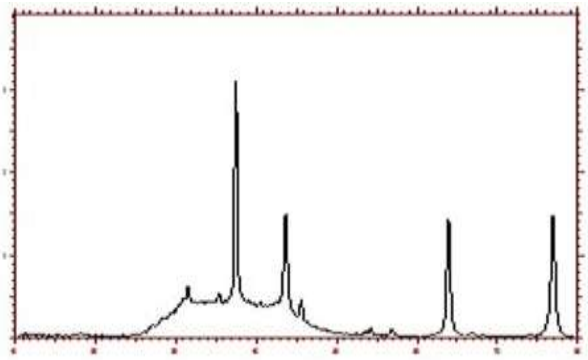

Fig 3. Representing XRD profile of silver nanoparticles.

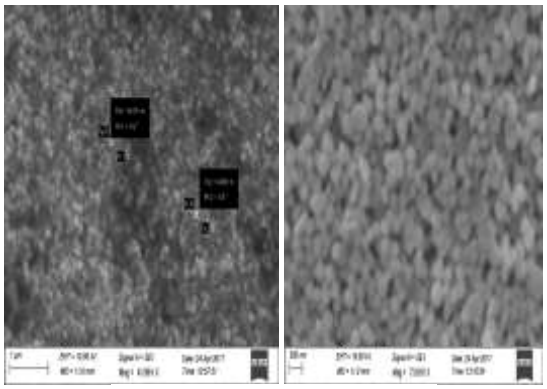

Fig 4: SEM image of AgNPs.

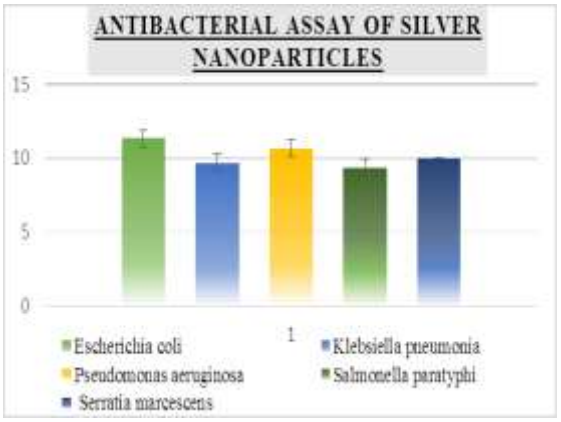

Fig 5: Antibacterial activity of Silver nanoparticles.

Table 1.

Bacterial strain	AgNPs
Escherichia coli	11.33±0.58
Klebsiella pneumonia	9.67±0.58
Pseudomonas aeruginosa	10.67±0.58
Salmonella paratyphi	9.33±0.58
Serratia marcescens	10±0

CONCLUSION

Green synthesis of AgNPs from the aqueous extract of *P. roxburghii* leaves was done by reduction method. It is cost effective and simple biological method. Reduction of $AgNO_3$ to Ag+ or Ag^o in the presence of aqueous extract of *P. roxburghii* was observed by change in color. Various techniques like UV-Vis, XRD, SEM and TEM were used to characterize synthesized nanoparticles. These results gives confirmation of reduction of AgNPs and also about the nature of particles i.e. size and shape. Inhibition zone against various bacteria in disc diffusion assay confirms the effectiveness of synthesized nanoparticles.

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